THE EFFECT OF SODIUM, POTASSIUM AND CALCIUM IONS ON CHANGES IN VOLUME OF AMOEBA PROTEUS[1]

S. O. MAST AND COLEEN FOWLER

(*From the Zoölogical Laboratory of the Johns Hopkins University*)

Introduction

McCutcheon and Lucké (1928) found that, with the same osmotic concentration, permeability of the eggs of *Arbacia* to water is greater in solutions of dextrose containing 0.01 M NaCl or KCl and less in solutions of dextrose containing 0.004 M $CaCl_2$ or $MgCl_2$ than in solutions of dextrose in distilled water. These results indicate that sodium and potassium ions increase and calcium and magnesium ions decrease permeability to water. Jacobs (1930) showed that hemolysis of erythrocytes in solutions of non-electrolytes, 0.01 M or 0.02 M, is almost as rapid as it is in distilled water; but that in solutions of electrolytes, e.g. NaCl 0.001 M or $CaCl_2$ 0.0001 M, it is considerably slower. These results indicate that sodium as well as calcium ions decrease permeability to water, i.e. that under some conditions monovalent as well as divalent salts cause decrease in permeability.

The experiments considered in the following pages were undertaken to ascertain the effect of sodium, potassium and calcium ions in phosphate buffer solutions (0.002 M, pH 6.8) on changes in the volume of *Amoeba proteus*.

Materials

Amoeba proteus was used exclusively in these experiments. It was grown in cultures prepared according to the method described in an earlier paper (Mast and Fowler, 1935). The distilled water used was triple redistilled from a tandem glass still (Mast, 1928). The solutions used were prepared by making two stocks, one containing the primary phosphate of the cation to be tested, 0.002 M, and the other containing the hydroxide of the same cation, 0.002 M, and mixing the two stocks

[1] This paper is the second in a series on the relation between environmental factors and water-content in *Amoeba proteus*. The first paper in the series, entitled "Permeability of Amoeba proteus to Water," was published in the *Journal of Cellular and Comparative Physiology*, Vol. 6, pp. 151–167, 1935.

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in the proportions necessary to maintain the hydrogen ion concentration at pH 6.8. The preparation and standardization of the hydroxide stock solutions is described in the paper cited above. All the solutions thus prepared contained the same concentration of the metallic cation used and the osmotic concentration of these solutions was practically the same as that in which the amoebae were grown, i.e. modified Ringer solution.

If the salts in the solutions used had been completely ionized, the osmotic concentration of the solutions containing calcium would have been greater than those containing either sodium or potassium because if $CaH_4(PO_4)_2$ ionizes there are produced two phosphate ions for each

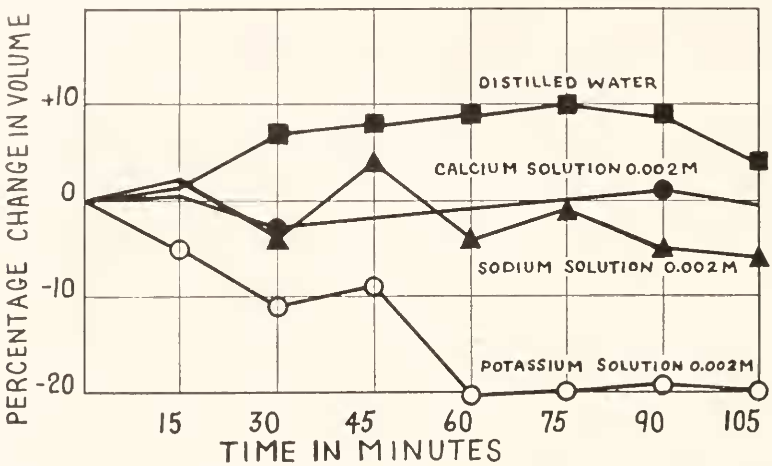

FIG. 1. Graphs showing the relation between sodium, potassium and calcium respectively in phosphate buffer solutions (pH 6.8) and changes in volume in *Amoeba proteus* transferred from dilute Ringer solution of the same osmotic concentration. Each point on the curves represents the average of the results of three measurements made on three different individuals. Ordinates, change in volume as percentage of the original volume; abscissæ, time in minutes after transfer from dilute Ringer solution.

calcium ion, whereas if KH_2PO_4 or NaH_2PO_4 ionizes there is produced only one phosphate ion for each potassium or sodium ion. At the concentration used, however, the sodium and the potassium compounds were slightly more ionized than the calcium compounds. The number of ions per unit volume and the osmotic pressure were probably therefore practically the same in the solutions used.

Methods and Results

Three methods were used in ascertaining the effect of these solutions on changes in volume of *Amoeba*.

1. Six amoebae were prepared, selected, and measured individually in modified Ringer solution as described in the paper cited above (Mast and Fowler, 1935). Then they were transferred to distilled water and measured at fifteen-minute intervals. Then this was repeated with other individuals, using in place of distilled water phosphate buffer solutions which contained sodium, potassium and calcium respectively, but with only three individuals in each. The results obtained are presented in Fig. 1.

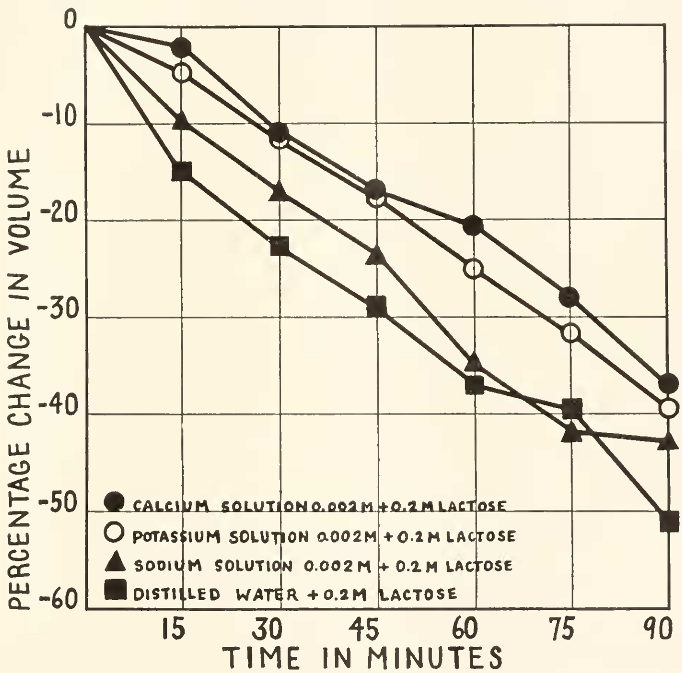

FIG. 2. Graphs showing the relation between sodium, potassium and calcium respectively in hypertonic solutions of lactose (pH 6.8) and changes in volume in *Amoeba proteus* transferred from dilute Ringer solution.

Each point on the curves represents the average of the results of ten measurements made on 7 to 12 different individuals. Ordinates, change in volume as percentage of the original volume; abscissæ, time in minutes after transfer.

Figure 1 indicates that the amoebae transferred from modified Ringer solution to distilled water increased 10 per cent in volume in 75 minutes; that those transferred to the calcium phosphate buffer solutions of practically the same osmotic concentration as the Ringer solution, did not change appreciably in volume during the time represented in the graph, and that those transferred to the sodium phosphate buffer solution decreased only slightly if at all during this time, but that those transferred to the potassium phosphate buffer solution of the same osmotic concentration as the preceding solutions, decreased 20 per cent in 60 minutes and then remained constant.

After the experiment had continued 105 minutes, the amoebae in the distilled water and in the calcium phosphate buffer solution were normal and they continued to live for several days; those in the sodium phosphate buffer solution soon began to round up and decrease in volume and they disintegrated about 60 minutes later; and those in the potassium buffer solutions disintegrated even sooner. However, those in all the solutions recovered fully in culture solution, if they had not been in the salt solutions more than one hour. The results obtained show, therefore, that without any change in osmotic concen-

TABLE I

Percentage changes in volume in amoebae transferred from modified Ringer solution (0.002 M, pH 6.8) to a solution containing sodium as the only metallic cation, of the same hydrogen ion and osmotic concentration as the Ringer solution, plus 0.2 M lactose. Temperature, 22.7°–25.5°; E_m, probable error.

Designation of specimen	Time in minutes after transfer						
	15	30	45	60	75	90	105
1	− 7	−17	−17	−27	−32	−32	−46
2	− 2	−18	−37	−48	−55	−60	−55
3	−13	−20	−25	−29	−34	−46	−46
4	−14	−23	−26	−40	−53	−52	−62
5	− 8	−24	−45	−61	−63		
6	−17	−20	−28	−26	−33	−35	−43
7	+ 1	− 9	−21	−31	−36	−41	−44
8	−16	− 9	− 7	−41	−36	−27	−27
9	−18	−26	−23	−34	−41	−39	−51
10	− 3	− 5	− 8	−16	−41	−58	
Mean	−10	−17	−23	−35	−42	−43	−47
E_m	±1.382	±1.442	±2.36	±2.58	±2.18	±2.44	±2.28

tration potassium causes rapid loss of water in *Amoeba*, without any appreciable injury; and that sodium and calcium have little if any effect.

2. Several amoebae were prepared, selected, and measured as in method 1, and then transferred to 0.2 M lactose in distilled water, and measured at fifteen-minute intervals. Then this was repeated with phosphate buffer solutions which contained lactose (0.2 M) and sodium, potassium and calcium (0.002 M) respectively. The results obtained are presented in Fig. 2 and Tables I, II, and III.

Figure 2 indicates that the amoebae which were transferred from modified Ringer solution to hypertonic solutions, consisting of lactose in distilled water and respectively sodium, potassium and calcium plus lactose, decreased in volume in all the solutions, but that the decrease was least in the solution containing calcium and greatest in distilled water plus lactose.

Tables I, II, and III show that there was marked individual variation in the reduction in the volume of the amoebae in all the hypertonic solutions tested, but that in spite of this variation, the probable error is relatively so small that there is overlapping at only a few points on the curves. This practically proves that the differences in the means of the reduction in volume in the different solutions, indicated in the

TABLE II

Percentage changes in volume in amoebae transferred from modified Ringer solutions (0.002 M, pH 6.8) to a solution containing potassium as the only metallic cation, of the same hydrogen ion and osmotic concentration as the Ringer solution, plus 0.2 M lactose. Temperature, 22.1°–25.2°; E_m, probable error.

Designation of specimen	Time in minutes after transfer						
	15	30	45	60	75	90	105
1	−2	−8	−19	−23	−28	−23	−36
2	−10	−21	−24	−30	−28	−35	−33
3	+10	−11	−16	−23	−35	−43	
4	−2	−10	−15	−15	−28	−20	−34
5	−21	−14	−22	−31	−24	−43	−43
6	−11	−17	−23	−32	−42	−57	
7	−11	−14	−11	−30	−37	−43	
8	+10	−4					
9	−4	−4	−11	−29	−37		
10	−17	−17	−17	−20	−20	−46	
11	−9	−9	−16	−20	−34	−43	
12	−11	−11	−18	−24	−32	−42	−58
Mean	−6	−12	−18	−25	−32	−40	−41
E_m	±1.79	±0.97	±0.85	±1.07	±1.26	±2.10	±2.80

curves in Fig. 2, are significant and consequently that the order of effectiveness on preventing loss of water in *Amoeba*, is Ca>K>Na, with very little difference between calcium and potassium.

The facts that the osmotic concentrations of the solutions which contained respectively sodium, potassium and calcium plus lactose were practically equal and that the osmotic concentration of these solutions was somewhat higher than that of the distilled water plus lactose, show that the monovalent sodium and potassium ions as well as the divalent calcium ions, retard the loss of water, but that calcium ions are more effective than sodium or potassium ions.

3. Ten amoebae were prepared, selected, and measured in modified Ringer solution as in methods 1 and 2, then they were transferred to this solution containing lactose (0.2 M), left 30 minutes and measured again. They were then transferred to distilled water and measured at 30-minute intervals. This whole process was then repeated with other specimens in respectively sodium, potassium and calcium phosphate buffer solutions (0.002 M) in place of distilled water. The results obtained are presented in Fig. 3.

Figure 3 shows that the amoebae which were transferred from modified Ringer solution to modified Ringer solution containing lactose (0.2 M) decreased in volume to about 70 per cent of the original volume in 30 minutes and that after they had been transferred from this solution to distilled water or to phosphate buffer solutions containing

TABLE III

Percentage changes in volume in amoebae transferred from modified Ringer solution (0.002 M, pH 6.8) to a solution containing calcium as the only metallic cation, of the same hydrogen ion and osmotic concentration as the Ringer solution, plus 0.2 M lactose. Temperature, 22.5°–24.0°; E_m, probable error.

Designation of specimen	Time in minutes after transfer						
	15	30	45	60	75	90	105
1	− 8	− 2	−11	−20	−23	−29	
2	−12	−22	−25	−17	−29	−44	−53
3	−12	−21	−23	−26	−41		
4	+ 7	− 8	− 6	−13	−30	−36	−43
5	+ 4	− 5	− 8				
6	+ 7	−18	−27	−29	−32	−38	−47
7	− 3	− 5	−21	−17	−13	−42	
Mean	− 2	−12	−17	−21	−28	−38	−48
E_m	±2.01	±1.98	±2.01	±1.53	±1.93	±1.55	±1.61

sodium, potassium or calcium, they increased in volume, but that the increase was much more rapid and much greater in distilled water than in any of the salt solutions. The figure shows that in all the salt solutions the amoebae increased to a maximum and then decreased, but that increase was greater in the solution which contained sodium than those which contained potassium or calcium, the maximum increase being 22 per cent for sodium and only 9 per cent for potassium and 4 per cent for calcium. It also shows that in distilled water the amoebae nearly regained their original volume in 30 minutes, and then continued to increase as long as measurements were made, i.e. for 90 minutes, and that at the close of the experiment they were 17 per cent larger than at the beginning.

The individual variations in the increase in volume in the hypotonic solutions were essentially the same as those recorded in Tables I, II and III for hypertonic solutions, but the differences in the means were greater so that there was no overlapping of the probable errors, except at the last points on the curves. The differences in the means of the increase in volume in the different solutions indicated in the curves in Fig. 3, are therefore significant, showing that the order of

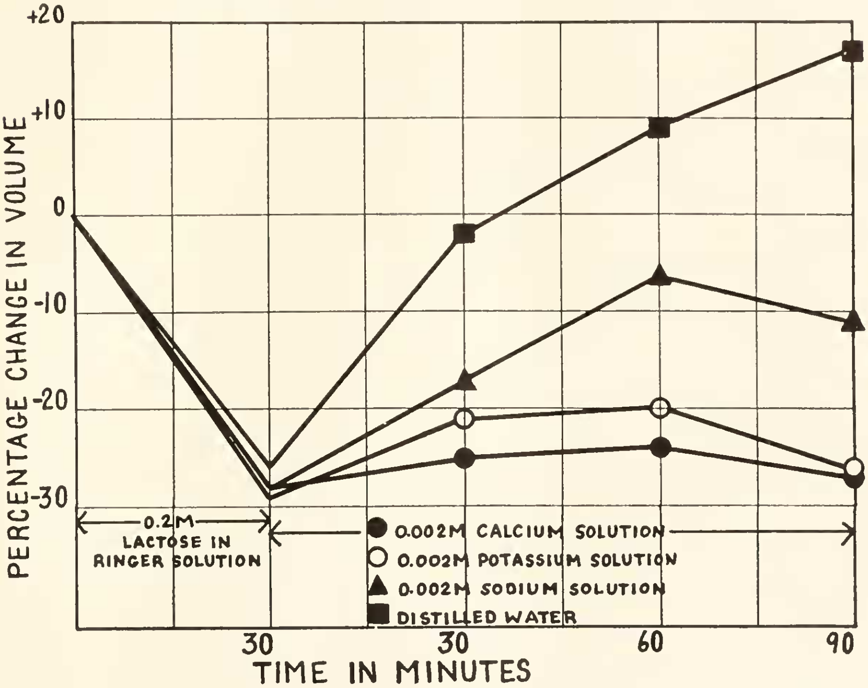

FIG. 3. Graphs showing the relation between sodium, potassium and calcium respectively in hypotonic buffer solutions (pH 6.8) and changes in volume in *Amoeba proteus* transferred from weak Ringer solution containing 0.2 M lactose.

Each point on the curves represents the average of the results obtained in ten measurements made on ten different individuals. Ordinates, change in volume as percentage of the original volume; abscissæ, time in minutes after transfer.

effectiveness on gain of water in *Amoeba* in hypotonic solutions is, Ca > K > Na, that is, that it is the same as it is on loss of water in hypertonic solutions.

By comparing Fig. 3 with Fig. 1, it will be seen that if *Amoeba* is transferred from Ringer solution (0.002 M) to potassium phosphate solution (0.002 M) it decreases markedly in volume, but that if it is transferred from lactose solution (0.2 M) to potassium phosphate solution (0.002 M) it increases somewhat. This difference is doubtless correlated with the fact that the water-content was much higher in the amoebae which were transferred from the modified Ringer solution than it was in those which were transferred from the lactose solution.

The results presented in Fig. 3 show therefore that monovalent as well as divalent cations retard the entrance of water but that, just as in reference to loss of water, the latter are more effective than the former; and they support the conclusion reached above that both monovalent and divalent metallic cations tend to maintain the water-content in *Amoeba* constant.

Discussion

The question now arises as to how the metallic ions act in their tendency to maintain the water-content of *Amoeba* constant.

Under normal conditions much water is eliminated by the contractile vacuole and considerable water enters with the food in the process of feeding (Mast and Hahnert, 1935) and some possibly enters in the formation of cups without food (Mast and Doyle, 1934). It may be then that the metallic ions influence the water-content of *Amoeba* through action on some or all of these processes. Such action could, however, not have been seriously involved in the results presented in the preceding pages; for in the solutions used there was, during the time the observations were made, no feeding and no formation of cups of any sort and the contractile vacuole did not function except in a few of the more dilute solutions and then only after the amoebae had been in the solution half an hour or more. The changes in water-content observed must therefore have been due to passage of water directly through the surface layer of the amoebae, and the effects of the metallic ions on the rate of the passage through this layer must have been due either to their action on the permeability of this layer or to their action on the osmotic concentration of substances in the amoebae, or to their action on imbibition, but it is probably largely due to the first. This matter will be discussed more fully in a subsequent paper.

The conclusion that monovalent as well as divalent metallic cations tend to maintain the water-content of *Amoeba* constant, is in agreement with the results obtained by Jacobs, who showed that in erythrocytes permeability to water is decreased by the addition of either NaCl or $CaCl_2$ to distilled water; but they are not in agreement with those obtained by McCutcheon and Lucké, who showed that permeability of the eggs of *Arbacia* to water is increased if NaCl or KCl is added to a solution of dextrose in distilled water and decreased if $CaCl_2$ or $MgCl_2$ is added.

Summary

1. If amoebae are transferred from modified Ringer solution (0.002 M, pH 6.8) to distilled water or to phosphate buffer solution containing respectively sodium, potassium and calcium as the only metallic cation (0.002 M, pH 6.8), they increase in size in distilled water, decrease in size in the solution which contains potassium and remain nearly the same in size in the solution which contains sodium or calcium.

2. If they are transferred from modified Ringer solution (0.002 M, pH 6.8) to distilled water containing lactose (0.2 M) or to phosphate buffer solutions containing lactose (0.2 M) and respectively sodium, potassium and calcium as the only metallic cation, they decrease in volume in all the solutions but they decrease most rapidly in distilled water containing lactose, less rapidly in the solution containing sodium and lactose, still less rapidly in the solution containing potassium and lactose, and least rapidly in the solution containing calcium and lactose.

3. If they are transferred from modified Ringer solution (0.002 M, pH 6.8) to Ringer solution containing lactose (0.2 M) and left 30 minutes, they decrease greatly in volume. If they are then transferred respectively to distilled water and to phosphate buffer solutions, containing respectively sodium, potassium and calcium (0.002 M, pH 6.8), they increase in volume in all the solutions, but they increase most rapidly in distilled water, less rapidly in the solution containing sodium, still less rapidly in the solution containing potassium and least rapidly in the solution containing calcium.

4. These results show that under some conditions monovalent as well as divalent cations cause decrease in the rate at which water passed into and out of *Amoeba proteus*. This is probably largely due to their action on permeability to water.

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